EFFECT OF HYDROCORTISONE ON SOME INDICES OF ENERGY METABOLISM OF THE ERYTHROID AND MYELOID CELLS OF RABBIT BONE MARROW

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Administration of hydrocortisone to rabbits with the bone marrow in a state of erythroid hyperplasia led to an increase in the oxygen absorption, a slight decrease in the glucose absorption, an increase in the hexokinase activity, and a decrease in the rate of glycolysis and in the concentration of readily hydrolyzed phosphorous of high-energy compounds in the myelokaryocytes, consisting mainly of erythroblastic cells. During the investigation of myelokaryocytes obtained from bone marrow with reduced erythropoiesis and consisting mainly of myeloid cells, administration of the hormone was also followed by an increase in the oxygen absorption, but the glucose absorption and hexokinase activity were sharply reduced, the rate of glycolysis was unchanged, and the content of phosphorus of high-energy compounds was increased.

Administration of hydrocortisone greatly reduces the hexokinase activity of the bone marrow myelokaryocytes [1]. However, investigation of the effect of hydrocortisone on total glucokinase activity of the bone marrow cells of intact animals does not give a sufficiently clear idea of the action of the hormone because of the heterogeneity of the cells.

Recent work [2] has shown that the erythroblastic cells of the bone marrow differ from myeloid cells in the character of their energy metabolism. The former have high respiratory activity, while the latter are more dependent on carbohydrate utilization (glycolysis).

This paper describes an investigation of the effect of hydrocortisone on some aspects of the energy metabolism of myelokaryocytes obtained from bone marrow with reduced erythropoiesis and consisting mainly of myeloid cells, and also from bone marrow in a state of erythroid hyperplasia and containing mainly cells of the erythroblastic series.

EXPERIMENTAL METHOD

Chronic experiments were carried out on chinchilla rabbits weighing 2-3.5 kg. Hyperplasia of the erythropoietic tissue was induced by repeated bleeding, while reduction of erythropoiesis was induced by injections of a suspension of washed erythrocytes. After the development of hyper- or hypoplasia of the erythroid tissue (confirmed by myelogram counts), bone marrow was obtained by puncture from the femur, tibia, and ilium before and 15-16 h after injection of hydrocortisone. Hydrocortisone was given as a single intravenous or intramuscular injection in a dose of 50 mg/kg body weight. Myelokaryocytes were isolated by the hemolytic method [3]. The glucose concentration for the investigation of its absorption by the myelokaryocytes and of the hexokinase activity in extracts from them by Long's method [8], was determined by Nelson's method [10], and protein after incubation of the myelokaryocytes and their extracts was precipitated by Somogyi's method [12]. The oxygen consumption was measured manometrically in a Warburg's apparatus and the lactic acid content was determined by the method of Barker and Summerson [5]. The

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TABLE 1. Effect of Hydrocortisone on Some Indices of Energy Metabolism of Myelokaryocytes Obtained From Bone Marrow in a State of Erythroid Hyperplasia and From Bone Marrow with Reduced Erythropoiesis (M ± m)

Source of myelokaryocytes	Absorption of glucose (in μg/mg protein/h)		Hexokinase activity (in μg/mg protein/h)		Increase in lactic acid (in mg/10^9 cells/h)		Absorption of oxygen (in μl/10^9 cells/h)		Glycogen (in mg/10^9 cells)		Phosphorus of high-energy compounds (in μg/mg protein)	
	Initial	Hydrocortisone	Initial	Hydrocortisone	Initial	Hydrocortisone	Initial	Hydrocortisone	Initial	Hydrocortisone	Initial	Hydrocortisone
Bone marrow in state of erythroid hyperplasia	138±18 (25)	95±9 (17)	80±8,6 (34)	108±10,6 (17)	2,9±0,4 (10)	2,0±0,1 (10)	640±40,6 (10)	913±29,6 (10)	1,29±0,2 (10)	1,23±0,2 (10)	25±2 (21)	16±3 (7)
P	0,04		0,05		<0,04		<0,001		0,5		0,02	
Bone marrow with reduced erythropoiesis	283±50 (18)	36±10 (22)	124±10,7 (18)	60±10,6 (24)	4,3±0,4 (8)	5,1±0,6 (8)	367±17,2 (10)	727±41,3 (10)	2,2±0,2 (10)	1,6±0,1 (10)	14±1,3 (7)	20±3 (5)
P	<0,001		<0,001		0,25		<0,001		<0,001		0,02	

Note. Number of tests in parentheses.

protein concentration in the suspensions of myelokaryocytes and in their extracts was determined by Lowry's method [9]. Inorganic phosphorus was determined [7] in the supernatants of myelokaryocytes after precipitation with TCA, and the readily hydrolyzed phosphorus of the high-energy compounds determined after hydrolysis for 10 min in 1 N HCl solution at 100°C. Glycogen was isolated by Good's method [6] and determined as glucose with thymol sulfate reagent [11]. The methods used were fully described previously [2]. Statistical analysis of the results was carried out by the method of direct and indirect differences [4].

EXPERIMENTAL RESULTS

Before the experiments began, a suspension of myelokaryocytes isolated from the rabbits' bone marrow contained 21 ± 4.2% of erythroblasts–normoblasts, 27 ± 4.6% of myeloblasts–myelocytes, and 38 ± 5.8% of metamyelocytes–polymorphs. After repeated bleedings the number of erythroblasts–normoblasts rose to 60 ± 4.4% ($P < 0.001$), the number of myeloblasts–myelocytes fell to 9 ± 2.9% ($P = 0.005$), and the number of metamyelocytes–polymorphs fell to 22 ± 4.2% ($P = 0.04$). After repeated transfusions of erythrocytes the number of erythroblasts–normoblasts fell to 5 ± 1% ($P = 0.002$), the number of myeloblasts–myelocytes rose to 57 ± 4.5% ($P < 0.001$), and the number of metamyelocytes–polymorphs fell to 20 ± 5.2% ($P = 0.03$).

The following conclusion can be drawn from analysis of the results given in Table 1. Injection of hydrocortisone caused a small (by 31%), but statistically significant decrease in the absorption of glucose by the myelokaryocytes, most of which were erythroid cells. Hexokinase activity in extracts from the myelokaryocytes after administration of the hormone increased by 34%, while the intensity of glycolysis decreased. The decrease in glycolysis, which was observed despite an increase in hexokinase activity, may be to some extent due to a decrease in the absorption of glucose by the erythroid cells which, in turn, could be caused by a decrease in the permeability of the cell membranes which is characteristic of the action of glucocorticoids. The glycogen content showed no significant changes after administration of the hormone. The content of readily hydrolyzed phosphorus fell considerably although the absorption of oxygen by the erythroid cells and, consequently, the intensity of their respiration were increased by approximately 1.5 times.

Investigation of the myelokaryocytes, when 77% of these cells consisted of cells of the myeloid series, showed that their absorption of glucose fell after administration of hydrocortisone was reduced by 8 times to a low level. Hexokinase activity was reduced by half. Nevertheless, the intensity of glycolysis did not differ significantly from its initial level. If the glycogen content in the myeloid cells was significantly reduced after administration of hydrocortisone, it can be postulated that the substrate of glycolysis in the myeloid cells after administration of hydrocortisone was glycogen and not glucose, the absorption of which was sharply reduced, evidently through a

decrease in the permeability of the cell membranes and a decrease in the activity of hexokinase, an enzyme concerned with glucose metabolism in the cell. Absorption of oxygen by the myeloid cells was doubled and the concentration of readily hydrolyzed phosphorus of high-energy compounds was signicantly increased.

The effects of hydrocortisone on the indices of energy metabolism of the erythroid and myeloid cells studied in these experiments were different. The hormone characteristically caused a sharp decrease in the absorption of glucose and in hexokinase activity in the myeloid cells. Since the proportion of myeloid cells in the bone marrow of intact animals, according to figures given by various authors, is 42-60%,it can be considered that the decrease in glucokinase activity of the bone marrow after administration of hydrocortisone is due to inhibition of the enzyme in cells of myeloid origin. Hydrocortisone induced activation of respiration in both erythroid and myeloid cells.

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